

**RESPONSE OF RAPID DECLINE AFFECTED COCONUT
(COCOS NUCIFERA L.) PALMS TO MICRO-NUTRIENTS
AND COMMON SALT**

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ABSTRACT

Coconut Rapid Decline (CRD) is a serious disorder of coconut palms (*Cocos nucifera* L.) in Sri Lanka. A substantial loss of crop is well evident due to this disorder. This study aims to determine the effect of micro-nutrients and common salt on the performance of CRD-affected palms. Fifteen-years-old, mild, moderate and severe CRD-affected palms in Makandura Research Station (MRS) of Coconut Research Institute were used for this study. Affected palms were treated with the micro-nutrients (100 ml of 0.35 % $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.72 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solution/palm, root feeding at bi-monthly intervals) or common salt (NaCl, 1 kg/palm/year, surface application in the manure circle). Untreated CRD-affected palms were used as the control. Leaf nutrient levels before, one and two years after treatment application were analyzed. Vegetative, reproductive and physiological parameters were measured during two years, after treatment application.

Pre-treatment analysis of leaves revealed that Nitrogen (N) and Phosphorus (P) contents were within the sufficiency range and Potassium (K) content was above the sufficiency range in CRD-affected palms. Magnesium (Mg), Calcium (Ca), Sodium (Na), Copper (Cu) and Zinc (Zn) contents of those palms were below the sufficiency range. The macro and micro-nutrient contents (except Mg) of apparently healthy palms, adjacent to CRD-affected palms were within the sufficiency range for coconut. Therefore, it is suggested that the CRD-affected palms suffer from deficiency of Ca, Na, Cu and Zn. There was a significant difference in Iron (Fe) content among three severity stages of CRD-affected palms before initial treatment application, Fe concentration increased with increasing severity.

Phosphorus and Ca contents significantly increased in micro nutrient and common salt treated palms after one year, compared to before treatment application and control. However, there was no significant increase in leaf Cu, Zn and Na levels, even after two years of the treatment application. The percentage of drooping fronds, size of the spadix, number of female flowers, number and size of nuts and circumference of the trunk were not improved in treated palms one year after treatment. Two years after the treatment,

number of total fronds and functional green fronds in the canopy of affected palms were increased by common salt application. The stomatal diffusive resistance of these palms was also reduced after two years showing an improvement in palm water status. The experiment is in progress to observe the recovery of symptoms.

INTRODUCTION

Coconut Rapid Decline (CRD) is a recently reported disorder of coconut (*Cocos nucifera* L.) in Sri Lanka. It was first observed in 1997 at Makandura Research Station (MRS) of the Coconut Research Institute (CRI), Lunuwila, Sri Lanka that was planted with improved cultivars (Tall x Tall and Ambakelle special) of Sri Lanka tall variety. Later same symptoms were reported from coconut estates that were planted with improved tall cultivars, in the Puttlam, Kurunegala and Gampaha districts. This disorder is seen in the coconut palms of 15 – 35 years of age. A substantial crop loss is well evident due to this deadly disorder, etiology is not yet known. Most diagnostic visual symptom of the CRD is the drooping and rapid drying of the middle and lower whorl fronds. With the initial drooping symptoms, the trunk begins to taper and the internode length progressively reduces. No abnormalities in the internal tissues of the trunk of the affected palms were observed at any stage of the syndrome. Similarly no abnormalities were observed in the root system of CRD-affected palms, but the formation of new roots following a rainy season was found to be less compared to healthy palms. The palm produces fewer nuts resulting in a gradual yield decline. The yield is reduced in 6 – 8 months and the death of the palm occurs within 2-3 years (Ranasinghe *et al.*, 2002). There was a significant reduction in vegetative, reproductive growth and physiological parameters of CRD-affected palms compared to healthy palms, except for Stomatal Diffusive Resistance (SDR) and Total Chlorophyll Content (TCC) of the palms (Wijeratne *et al.* 2002). The cause of the disorder and treatments for recovery are still unknown. This study aims to determine the effect of micro-nutrients and common salt on the performance of CRD-affected palms.

MATERIALS AND METHODS

Plant Material

Fifteen-years-old, CRD-affected coconut palms in MRS of Coconut Research Institute were used for this study. The affected palms were grouped into three categories based on the severity of symptoms determined by average number of drooping fronds and the extent of trunk tapering.

Severity class 1 (S_1 - incipient stage) –	Approximately 33 % of total fronds starts drooping no dried drooping fronds, tapering of trunk not visible
Severity class 2 (S_2 - moderate stage) -	Approximately 22 % of total fronds are drooping and 10% of total fronds are dried and drooping, tapering of trunk visible
Severity class 3 (S_3 - severe stage) –	All drooping fronds are fallen, highly tapered trunk

Twelve apparently healthy palms (H) and 12 affected palms per treatment were used for the study. Thus, the experiment was Completely Randomized Block Design with 12 replicates for both apparently healthy and affected palms.

Treatments

T_1 – Micro-nutrients (100 ml of 0.35 % $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.72 % $\text{Zn SO}_4 \cdot 7\text{H}_2\text{O}$ solution / palm, root feeding at bi-monthly intervals)

T_2 - Common salt (1kg/palm/year, surface application in the manure circle), annually

T_3 - Control

All the palms were treated with a basal dose of Urea at the rate of 800 g/palm/y, Muriate of Potash at the rate of 1600 g/palm/y, Eppawala Rock Phosphate at the rate of 600 g/palm/y and Dolomite at the rate of 2000 g/palm/y. Apparently healthy palms were also given the same treatments for comparison. To assess the progress of symptoms the following data were collected;

Determination of leaf nutrient content

Leaf macro-nutrients, N, P, K, Ca, Mg and Na and micro-nutrients, Fe, Mn, Cu and Zn levels in 14th frond (counting the youngest fully open leaf as one) of healthy and affected palms were determined before initial treatment one year and two years after treatments. Total nitrogen content was determined by Kjeldahl method (Manual of Analytical methods, 2001). Total P, K, Ca, Mg, Na, Fe, Mn, Cu and Zn were analysed by wet digestion method using HNO_3 and HClO_4 acid mixture, mixed at 1:4 ratio (Manual of Analytical Methods, 2001). Concentration of N and P content were determined by Auto Analyser (Auto Analyser 3, BRAN + LUBBE, Germany) and K, Ca, Mg, Na, Fe, Mn, Cu, Zn were by Atomic Absorption Spectrophotometer (GBC 904 AA, Australia). Boron content in leaf was determined by dry ashing method (Manual of Analytical Methods, 2001).

Measurement of vegetative and reproductive parameters

Canopy characteristics

Number of healthy, drooped and broken fronds in the canopy was recorded before initial treatment and at three monthly intervals, for two years.

Trunk characteristics

Trunk circumference at canopy level and one foot below the canopy level was recorded before initial treatment and thereafter at six monthly intervals for two years.

Nut and spadix characteristics

Nut weight, length and circumference of nuts were recorded before initial treatment, and at two monthly intervals for two years after treatment. Length and circumference of unopened spadix were recorded at six monthly intervals for two years.

Physiological and biochemical characteristics

Determination of rate of transpiration and stomatal diffusive resistance

Rate of transpiration and stomatal diffusive resistance were measured on the central leaflets from both sides of ninth frond (counting the youngest fully open leaf as one), between 9.00 a.m. and 1.00 p.m. with full sun, using the LI-1600 Steady State Porometer (LI-COR Inc, Lincoln, USA).

Estimation of total chlorophyll content (TCC)

Leaflets from both sides of ninth frond were sampled on ice, cut in to small pieces, and homogenized in Acetone using an electric crusher (Ultra turrax T-25, GMBH, West Germany). During crushing, sample tubes were kept on ice to prevent temperature increase within the test tube and evaporation of Acetone. The crushed samples were centrifuged at 3000 rpm for five minutes and the absorbance was measured at 645 nm and 663 nm using UV/VIS spectrophotometer (Shimadzu UV 160 – A, Japan.). Total chlorophyll content was calculated according to Arnon (1949).

Data analysis

Data was analyzed by ANOVA, using the GLM procedure of SAS.

RESULTS AND DISCUSSION

Leaf nutrient content

Pre-treatment analysis of leaf nutrients revealed that N (1.9 - 2.1 %) and P (0.11 - 0.13 %) contents of CRD-affected palms were within the sufficiency range for coconut palms. However, Mg (0.25 % - 0.35 %), Ca (0.35 % - 0.5 %), Na (0.4 %-), Cu (5 mg/kg) and Zn (30 mg/kg) contents of those palms were below the sufficiency range. But K (1.2 % - 1.5 %), Fe (40 mg/kg) and Mn (60 mg/kg) contents were above the sufficiency level (Fremond, 1966, Manciot *et al.*, 1979). There was no significant difference in leaf macro- (N, P, K, Ca, Na, Mg) and micro- (Fe, Mn, Cu and Zn) nutrient contents among the CRD-affected palms before the initial treatment (Table 1).

Table 1: Macro and micro-nutrient contents of the 14th frond of the CRD-affected palms before treatments

Treatment	Macro-nutrients (%)						Micro-nutrients (mg/kg)			
	N	P	K	Mg	Ca	Na	Fe	Mn	Cu	Zn
T ₁ - Micro-nutrient	2.02	0.139	1.74	0.153	0.27	0.053	113	164	3.07	16.73
T ₂ - Common salt	2.08	0.137	1.91	0.145	0.26	0.056	119	134	3.32	18.05
T ₃ - Control	2.00	0.148	1.80	0.148	0.27	0.062	128	138	3.48	19.96
Significance	ns	Ns	ns	ns	ns	Ns	ns	ns	ns	ns

ns- not significant

Pre-treatment analysis of leaf nutrients of apparently healthy palms grown adjacent to CRD-affected palms revealed that all macro and micro-nutrient contents (except Mg), were within the sufficiency range for coconut and there was no significant differences among the palms (data not shown). Therefore, it is suggested that the CRD-affected palms suffer from deficiency of Ca, Na, Cu and Zn. Further, there was a significant difference in Fe content among three severity stages of CRD-affected palms before the initial treatment. Iron (Fe) concentration of leaves increased gradually with increasing severity of CRD (Table 2). Barr (1993) has observed an antagonistic effect between Fe and Zn in plants. Therefore, it can be suggested that the accumulation of Fe may possibly suppress the uptake of Cu, Zn, Ca and Na in CRD-affected palms due to antagonistic effects.

Table 2: Fe concentration of 14th frond of CRD-affected palms in response to severity stages, before treatment

Severity stage	Fe (mg/kg)
S ₁ - Incipient stage	95
S ₂ - Moderate stage	102
S ₃ - Severe stage	151
Significance	*
LSD	38.85

LSD – Least Significant Difference, * $p \leq 0.05$

One year after initial treatment, P content of T₁ and T₂ palms and Ca content of T₁ palms were significantly higher than untreated (control) palms. However, there was no significant increase in N, K, Mg, Na and micro-nutrients (Fe, Mn, Cu, and Zn) in treated (T₁ and T₂) compared to control palms (Table 3).

Table 3: Macro and micro-nutrient contents of the 14th frond of the CRD-affected Palms one year after initial treatment

Treatment	Macro nutrients (%)						Micro-nutrients(mg/kg)			
	N	P	K	Mg	Ca	Na	Fe	Mn	Cu	Zn
T ₁ -Micro-nutrient	1.95	0.184	1.79	0.200	0.38	0.082	118	190	2.90	22.20
T ₂ - Common salt	1.87	0.194	2.12	0.182	0.29	0.073	130	153	2.25	14.30
T ₃ - Control	1.81	0.130	1.79	0.150	0.26	0.082	93	110	2.17	14.14
Significance	ns	***	ns	ns	*	ns	ns	ns	ns	ns
LSD	-	0.023	-	-	0.099	-	-	-	-	-

LSD – Least Significant Difference, * $p \leq 0.05$, *** $p \leq 0.001$, ns- not significant

One year after the treatment, the P, Mg and Ca contents in treated palms (both affected and healthy) were increased compared to the initial concentrations. The reason can be due to conditioning of the palm nutrient absorption as a result of the application of Cu, Zn and common salt. Whilst there was an increase in Zn concentration of Cu and Zn -treated palms, the Cu concentration was not improved after one year compared to the pre-treatment analysis. Instead, there was a drop in concentration of Cu after one year, for which the reason has to be elucidated. Ohler (1999) reported that the Zn level increased significantly with the application of P and Mg. Whereas in the case of N or K application, the rate was decreased. At the inception of the trial, Cu content in the CRD-affected palms were 3.07 - 3.48 mg/kg. One year after Copper Sulphate application, Cu content reduced to 2.17 – 2.90 mg/kg. This may probably due to a dilution effect by newly

formed plant matter. There was no significant increase in leaf Cu, Zn and Na levels two years after application of Cu, Zn and common salt.

The coconut palms absorb more Zn and Cu with increasing doses of N, P and K (Kamaladevi *et al.*, 1975). Therefore, continuous application of N, P and K fertilizers over the years without supplementing with micro-nutrients might have resulted a lower availability of Zn and Cu in the soil of CRD-affected palms. Furthermore, it is also possible that, due to high vegetative growth of leaves of coconut palms treated with fertilizer, the palms may show a low concentration of micro-nutrients due to dilution effect. Dolar *et al.*, (1971) also reported that N, P and K fertilization resulted in greater plant uptake of Cu, Zn and Mn.

Canopy characteristics

Two years after treatment, the highest number of total fronds and functional green fronds in the canopy was observed in common salt treated palms and it is significantly higher than micro-nutrient treated and control palms. However, the lowest number of functional green fronds and the total fronds were observed in micro-nutrient treated palms (Table 4).

Table 4 : Number of functional green fronds (FGF, non drooping) and total number of fronds (TF) in CRD-affected palms in response to different treatments two years after treatment application

Treatment	Number of fronds	
	TF	FGF
T ₁ - Micro-nutrients	15.54	14.68
T ₂ - Common salt	22.04	21.06
T ₃ - Control	20.04	18.15
Significance	***	***

*** $p \leq 0.001$

However, a significant difference in the percentage of green-drooped fronds in the canopy was not observed among the treatments, two years after initial treatment application.

Trunk characteristics

Significant difference in circumference of trunk at the canopy level were not observed among the treatments two years after initial treatment (data not shown).

Nut and Spadix characteristics

There was no significant difference in weight, length and circumference of nuts and the number of nuts and female flowers per bunch among the treatments one year after the initial treatment application (Table 5).

Table 5: Weight, length, circumference of the nuts, number of nuts, total number of female flowers per bunch, spadix length and spadix circumference of CRD – affected palms in response to different treatments, two year after the initial treatment

Treatments	Weight Per Nut (kg)	Nut length (cm)	Nut Circumference (cm)	No. of nuts per bunch	No. of female flowers per bunch	Spadix length (cm)	Spadix circumference (cm)
T ₁ -Micro- nutrient	1.36	21.61	36.59	2	10	53.97	15.26
T ₂ -Common Salt	1.82	25.48	42.76	4	13	67.39	17.41
T ₃ -Control	1.48	25.62	49.28	3	10	64.98	17.49
Significance	ns	ns	ns	Ns	ns	ns	ns

ns- not significant

Furthermore, significant difference in length and circumference of unopened spadix were not observed among the treatments one year after the initial treatment (Table 6).

Table 6: Length and circumference of unopened spadix in CRD – affected palms in response to different treatments after two years

Treatments	Spadix length (cm)	Spadix circumference (cm)
T ₁ - Micro- nutrient	53.97	15.26
T ₂ - Common Salt	67.39	17.41
T ₃ - Control	64.98	17.49
Significance	Ns	ns

ns- not significant

Physiological and biochemical characteristics

Transpiration, stomatal diffusive resistance and chlorophyll content

No significant difference in the chlorophyll content was observed among the treatments one year after initial treatment. There were significant variations in the rate of leaf transpiration and stomatal diffusive resistance among the treatments. The rate of transpiration of T₁ and T₂ palms was significantly higher than control palms one year after the treatment (Table 7). It explains that there is an improvement in the stomatal regulation of treated palms, enabling an increased gas exchange capacity, specially the rate of photosynthesis. Supporting the fact, the stomatal diffusive resistance of CRD – affected palms treated with common salt and micro-nutrients were lower than untreated palms one year after the initial treatment (Table 7).

Table 7: Total chlorophyll content rate of transpiration and stomatal diffusive resistance of CRD – affected palms in response to different treatments after one year

Treatments	Chlorophyll content (mg/g fresh wt)	Rate of transpiration ($\mu\text{g cm}^{-2} \text{s}^{-1}$)	Stomatal diffusive Resistance (s cm^{-1})
T ₁ - Micro- nutrient	2.67	0.56	67.76
T ₂ - Common Salt	2.59	0.61	66.17
T ₃ - Control	2.86	0.49	96.06
Significant	Ns	*	**

ns- not significant, * $p \leq 0.05$, ** $p \leq 0.01$

There was a significant increase in stomatal diffusive resistance (SDR) in CRD-affected palms compared to healthy palms (Wijeratne *et. al.*, 2002), and this increase in stomatal resistance with the CRD symptoms development may have direct effects on other physiological processes such as photosynthesis, water and nutrients transport causing subsequent death of the palm.

After two years, there was no significant difference in the rate of transpiration among treatments. However, there was a significant difference in stomatal diffusive resistance between treated and non-treated palms. Complementing the canopy observations the highest rate of transpiration was observed in palms treated with common salt revealing the improved water status (Table 8).

Table 8: Rate of transpiration and stomatal diffusive resistance of CRD-affected palms in response to different treatments, two years after initial treatment

Treatment	Rate of transpiration ($\mu\text{g cm}^{-2} \text{s}^{-1}$)	Stomatal diffusive Resistance (s cm^{-1})
T ₁ - Micro-nutrients	1.807	33.304
T ₂ - Common salt	2.194	28.437
T ₃ -Control	1.644	30.123
Significance	Ns	ns

ns- not significant

Abnormal stomatal closure in CRD affected palms may be one of the factors that leads to palm death, in that significantly increased stomatal resistance affects other processes such as photosynthesis and water and nutrient transport of those palms. The reduction in xylem transport in CRD-affected palms, as determined by the reduced Lithium transport to the canopy could be mainly attributed to the abnormal stomatal closure, and was supported by the evidence of increased stomatal resistance with the development of the

disorder (Ranasinghe and Wijeratne, unpublished). A similar reduction in stomatal conductance (increase in stomatal resistance) that coincide with the appearance of foliar symptoms was a consistent symptom of ash yellows in *Fraxinus americana*, of corn stunt in maize and of lethal yellowing in *Cocos nucifera*, all diseases caused by phytoplasma (Matteoni and Sinclair, 1983; Leon *et al.*, 1996; Martinez *et al.*, 2000). However, with the application of Cu, Zn and common salt, the CRD-affected palms seem to have 'conditioned' and started absorbing nutrients. Since chloride are also important in osmotic adjustment of stomatal guard cells in coconut, it can be suggested that common salt treatment has influenced to improve the cell turgidity and water relations of the CRD-affected palms (Jayasekara *et al.*, 1993)

CONCLUSION

There was a micro-nutrient deficiency (Cu and Zn) in CRD-affected palms. Two years after micro nutrient or common salt treatment, number of total fronds and functional green fronds in the canopy were increased by common salt application. The stomatal diffusive resistance of these palms was also reduced after two years showing an improvement in palm water status. The experiment is in progress to observe the recovery symptoms.

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